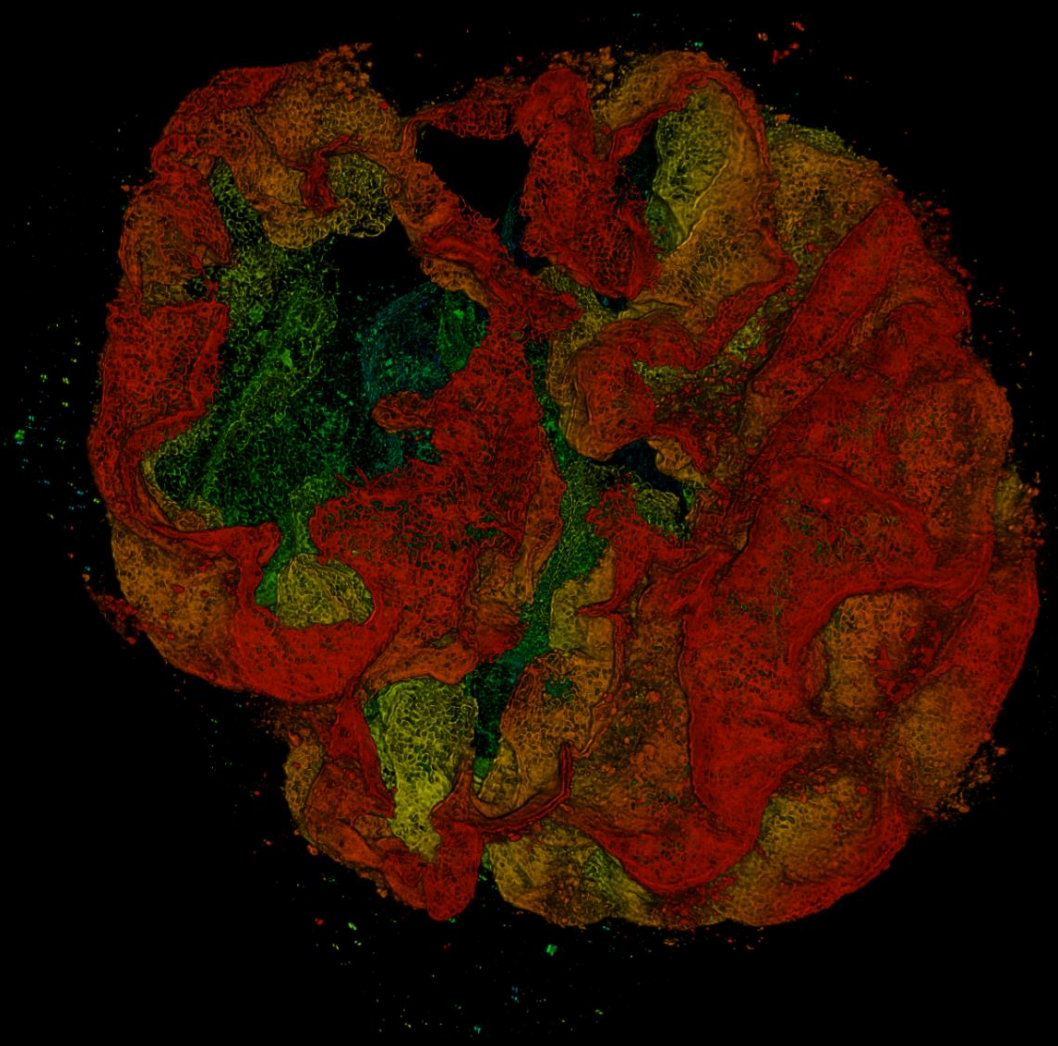
Super Resolution Microscopy: STED 3x



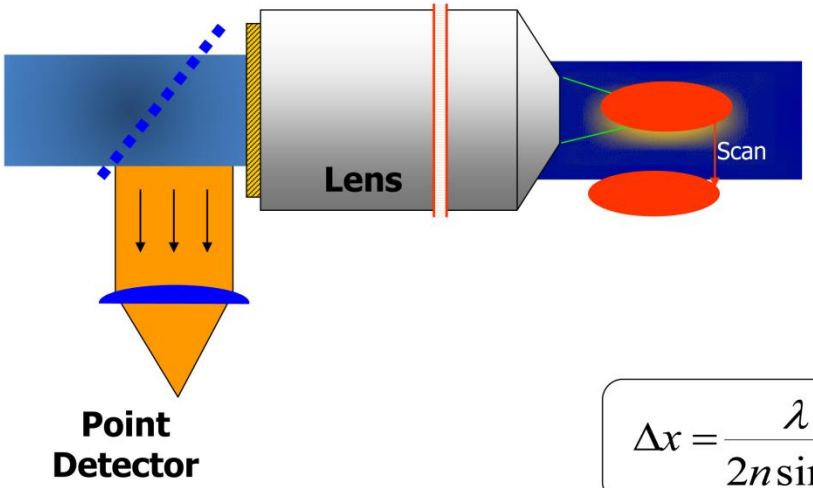
DENOThe Meeting 01/10/2019

Giulia Antonelli

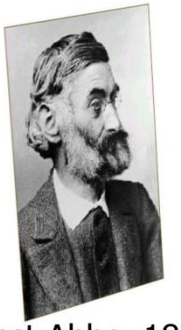
The resolution and diffraction limit

The **resolution** of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities.

The diffraction limit



$$\Delta x = \frac{\lambda}{2n\sin\alpha}$$



Ernst Abbe, 1873

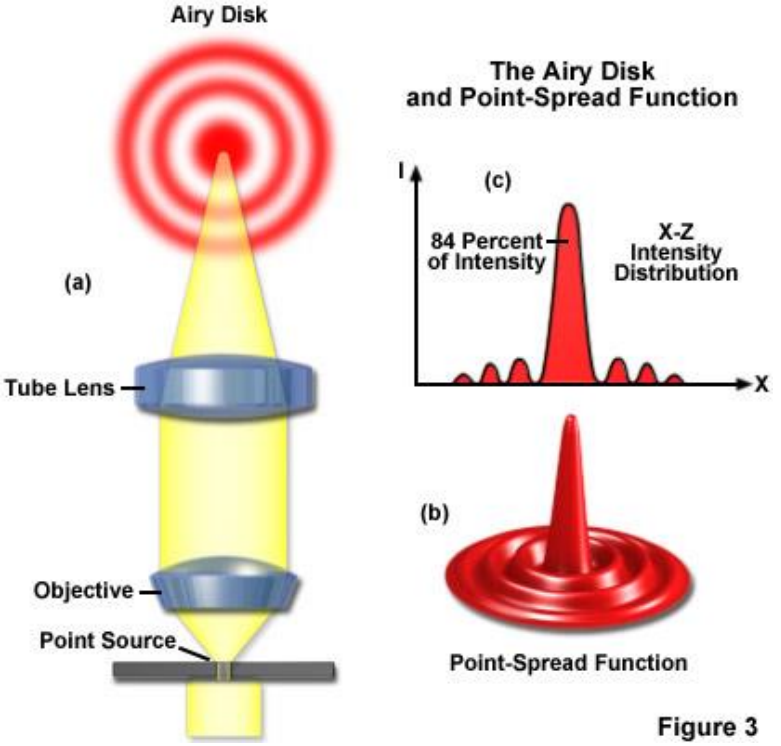
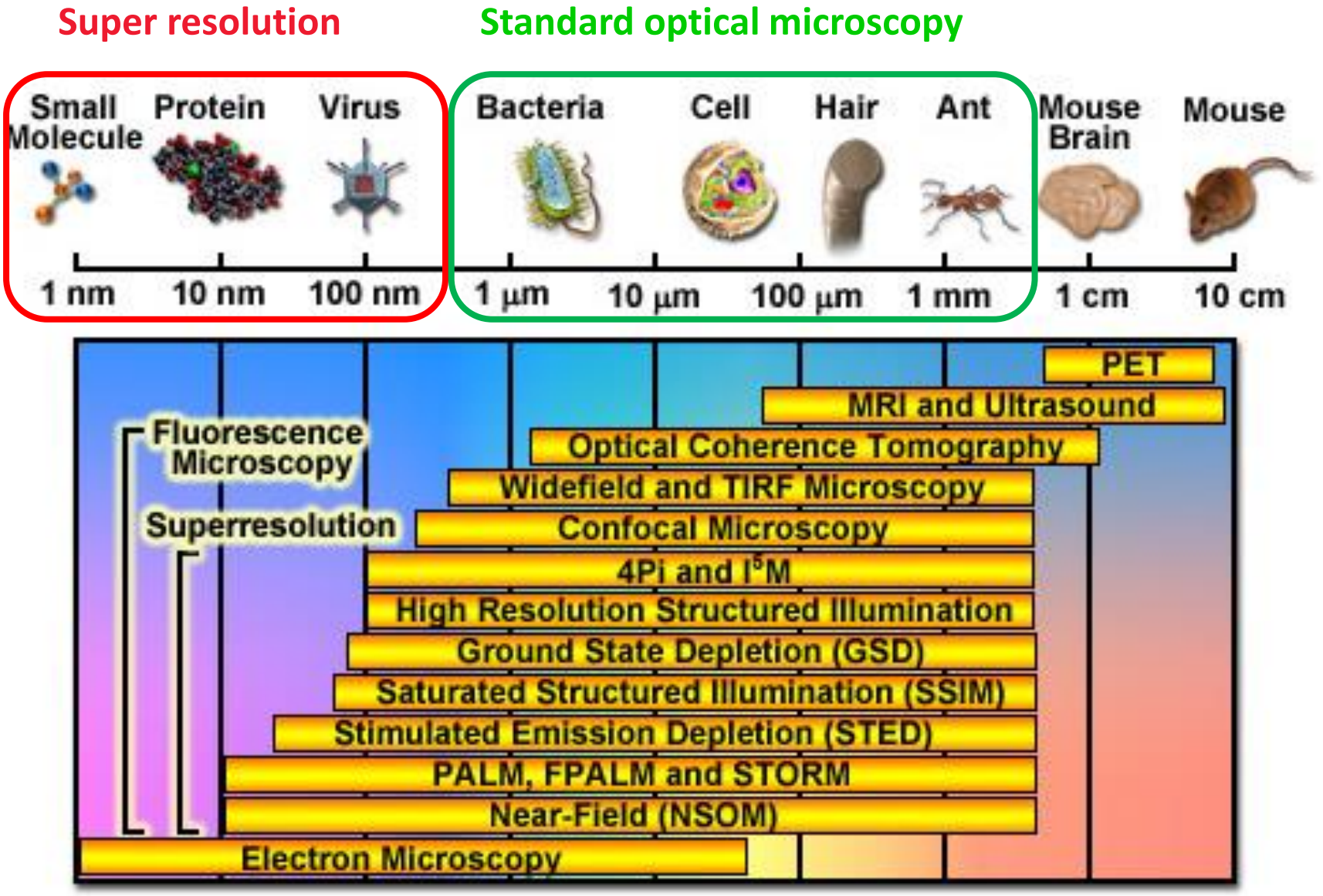


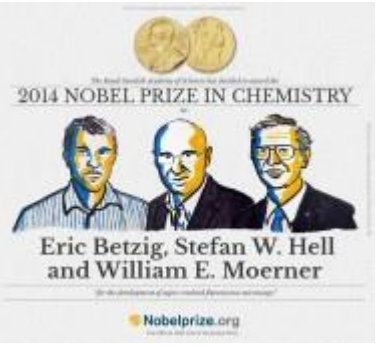
Figure 3

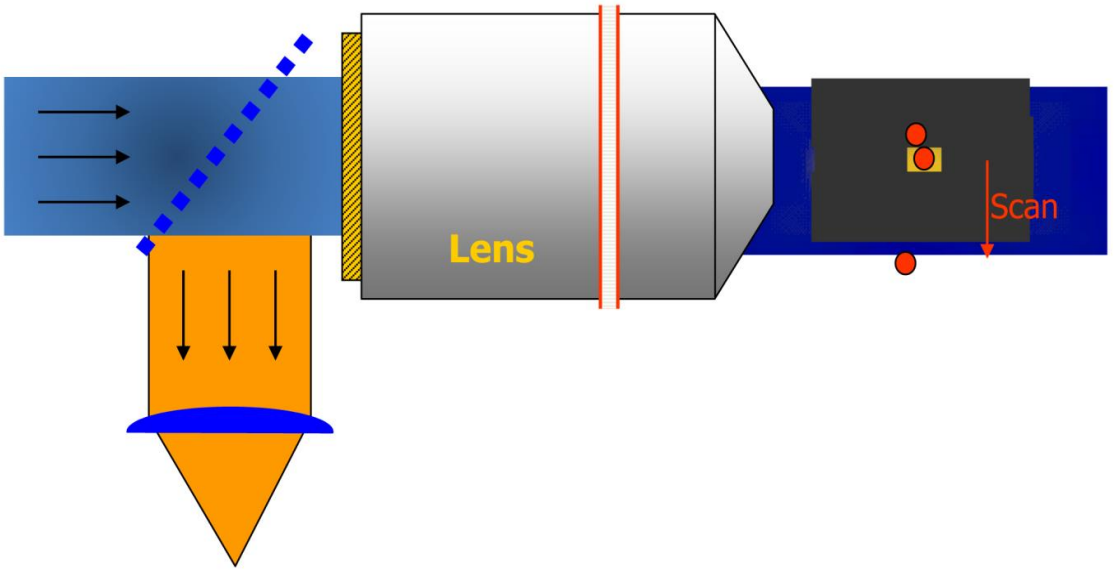
The resolution limit in optical microscopy is 200nm

Spatial resolution of biological imaging techniques

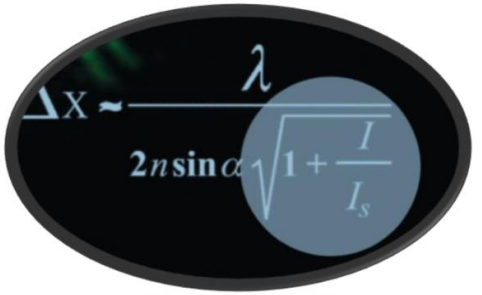


How to enhance resolution?
Super Resolution Microscopy



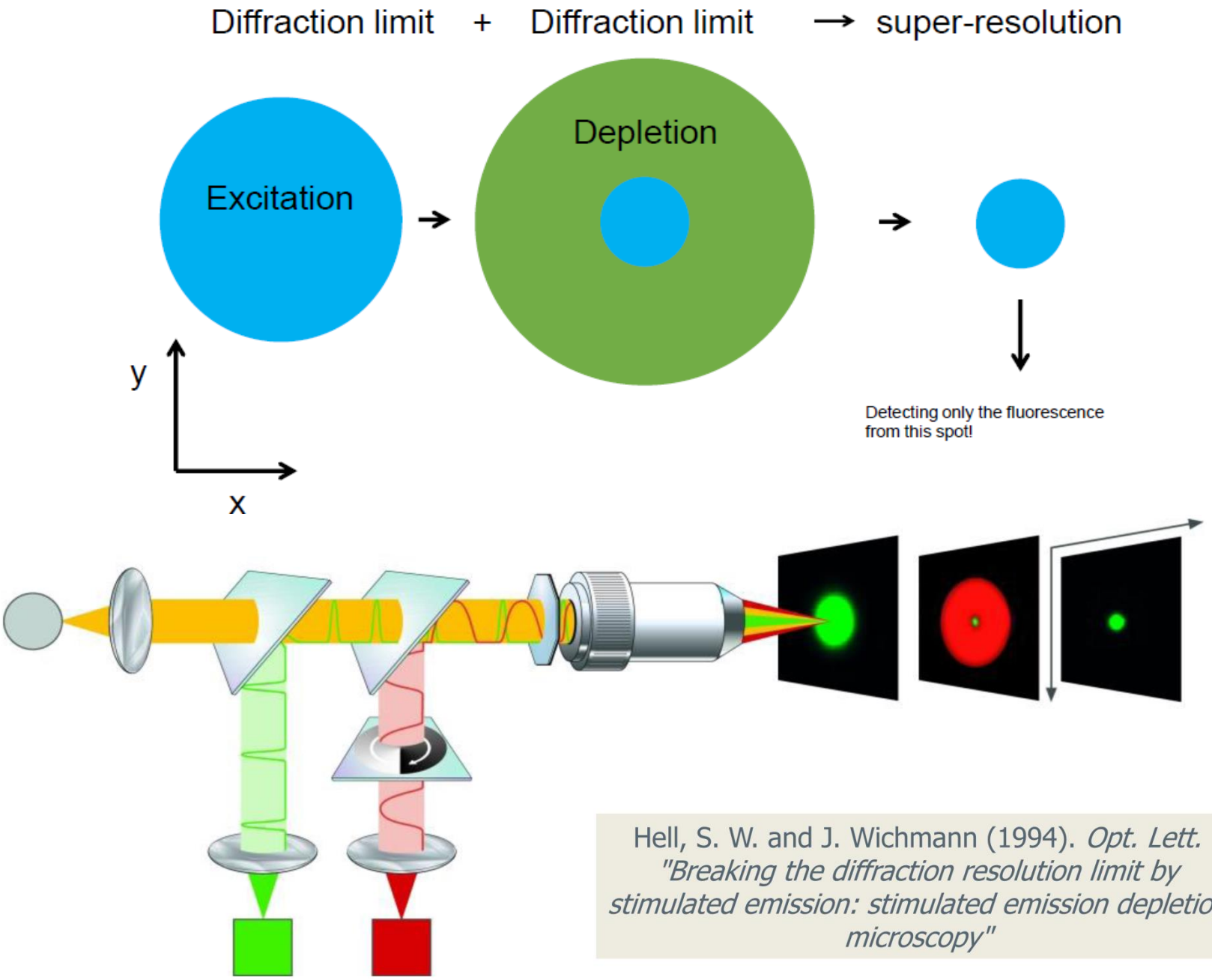


Stefan W. Hell,
Inventor of
STED-microscopy



Idea innovativa: **controllare l'emissione di fluorescenza dei fluorocromi in modo tale che molecole adiacenti più vicine di 200nm emettano in momenti diversi in modo da poter essere individuate come molecole distinte**

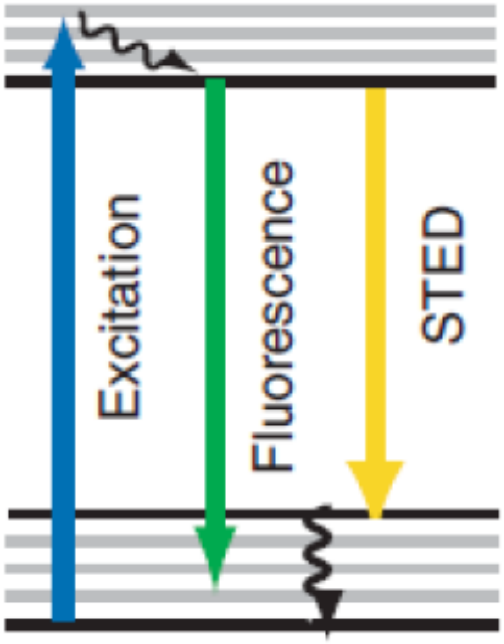
Stimulated Emission depletion (STED)

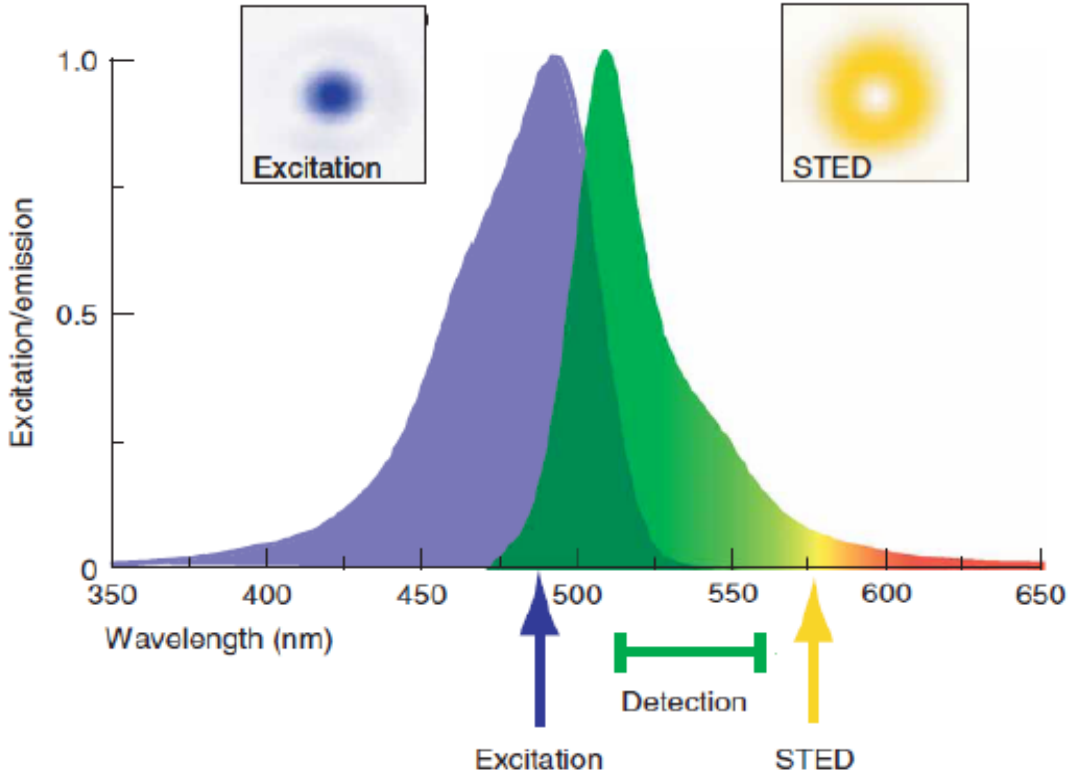


Hell, S. W. and J. Wichmann (1994). *Opt. Lett.* *"Breaking the diffraction resolution limit by stimulated emission: stimulated emission depletion microscopy"*

The resolution limit of STED microscopy is 30-50 nm

Stimulated Emission Depletion (STED)

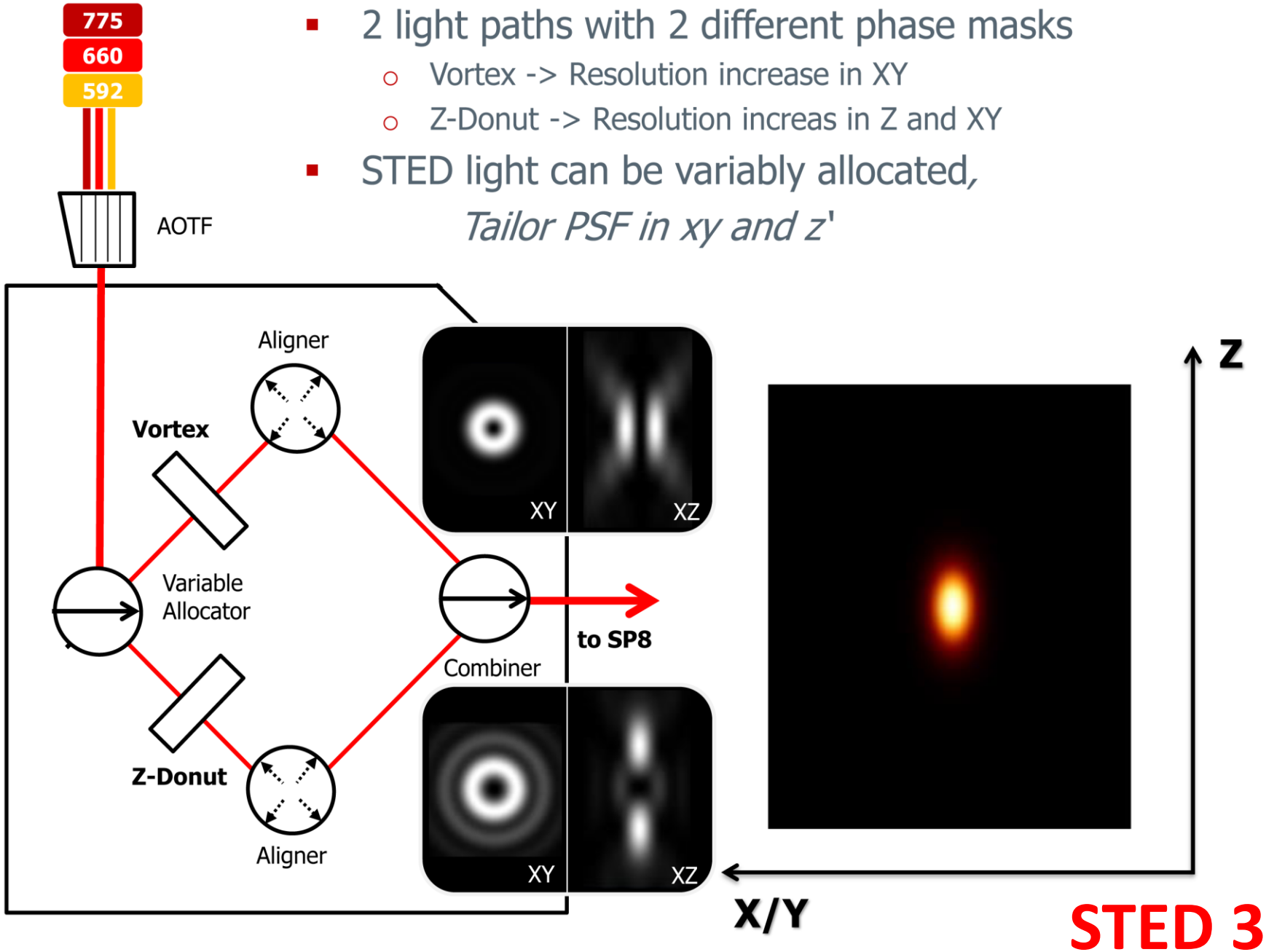




Push the boundaries of your science – in all dimensions
The new STED module

- 2 light paths with 2 different phase masks
  - Vortex -> Resolution increase in XY
  - Z-Donut -> Resolution increas in Z and XY
- STED light can be variably allocated,

  Tailor PSF in xy and z'



STED 3X

Three Different Objectives are available

	HC PL APO 100x/1.4 oil STED WHITE	HC PL APO 93x/1.3 Glyc motCORR STED WHITE	HC PL APO 86x/1.2 W motCORR STED WHITE
Magnification	100x	93x	86x
Free working distance	130 µm	300 µm	300 µm
Immersion medium	Oil	Glycerol*	Water
	Type F imm. n_e^{23}= 1.518	Type G imm. n_e^{23}= 1.45 Glycerine solution n_e^{37}= 1.46	Water
Application	Fixed cell samples	Deep tissue, fixed samples, live-cell	Live–cell, FCS

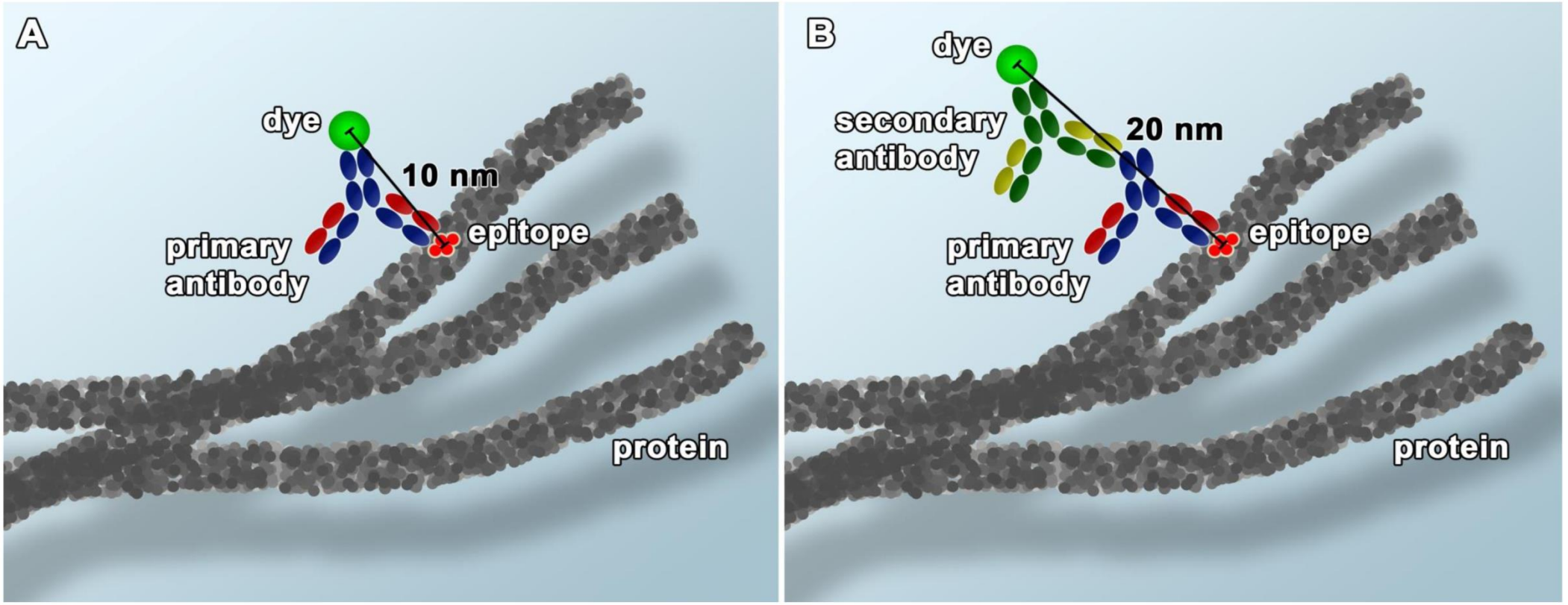
Advantages of STED microscopy

- Fast imaging
- Imaging *in vivo*
- Multicolored stainings
- Optical sectioning for 3D reconstructions
- Nanoscopic sub-cellular structures: resolution in xy 30-50 nm, resolution in z 100-120 nm

Sample preparation: the crucial points to obtain the best SR image

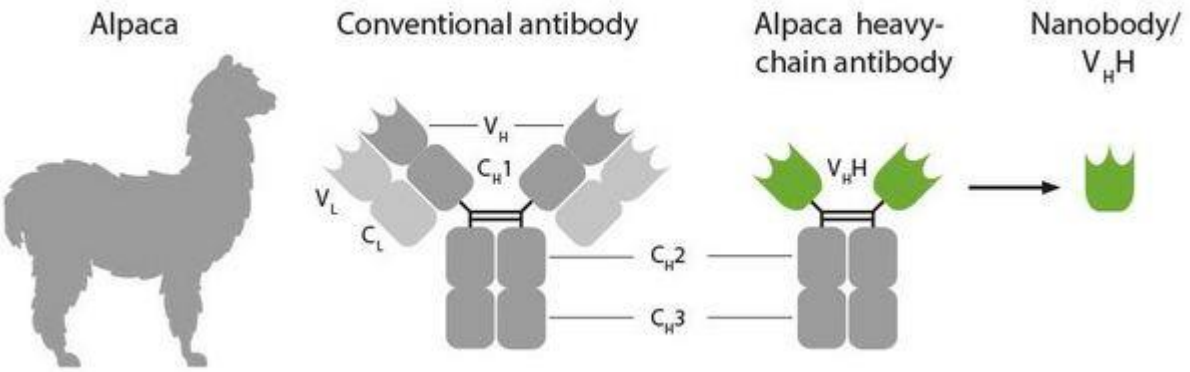
- Epitope/dye distance
- Labeling density
- Choice of fluorophores
- Mounting & Immersion
- Coverslip Thickness: 170 μm

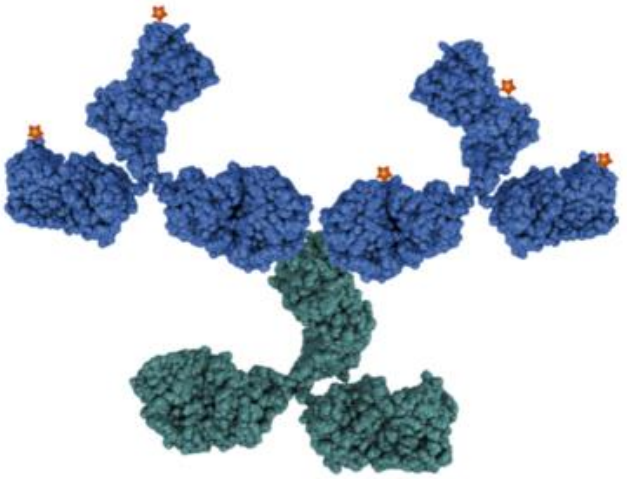
Epitope/dye distance



7 – 10nm
Fab fragments: 3.5 – 5.5nm

Nanobodies/VHHs





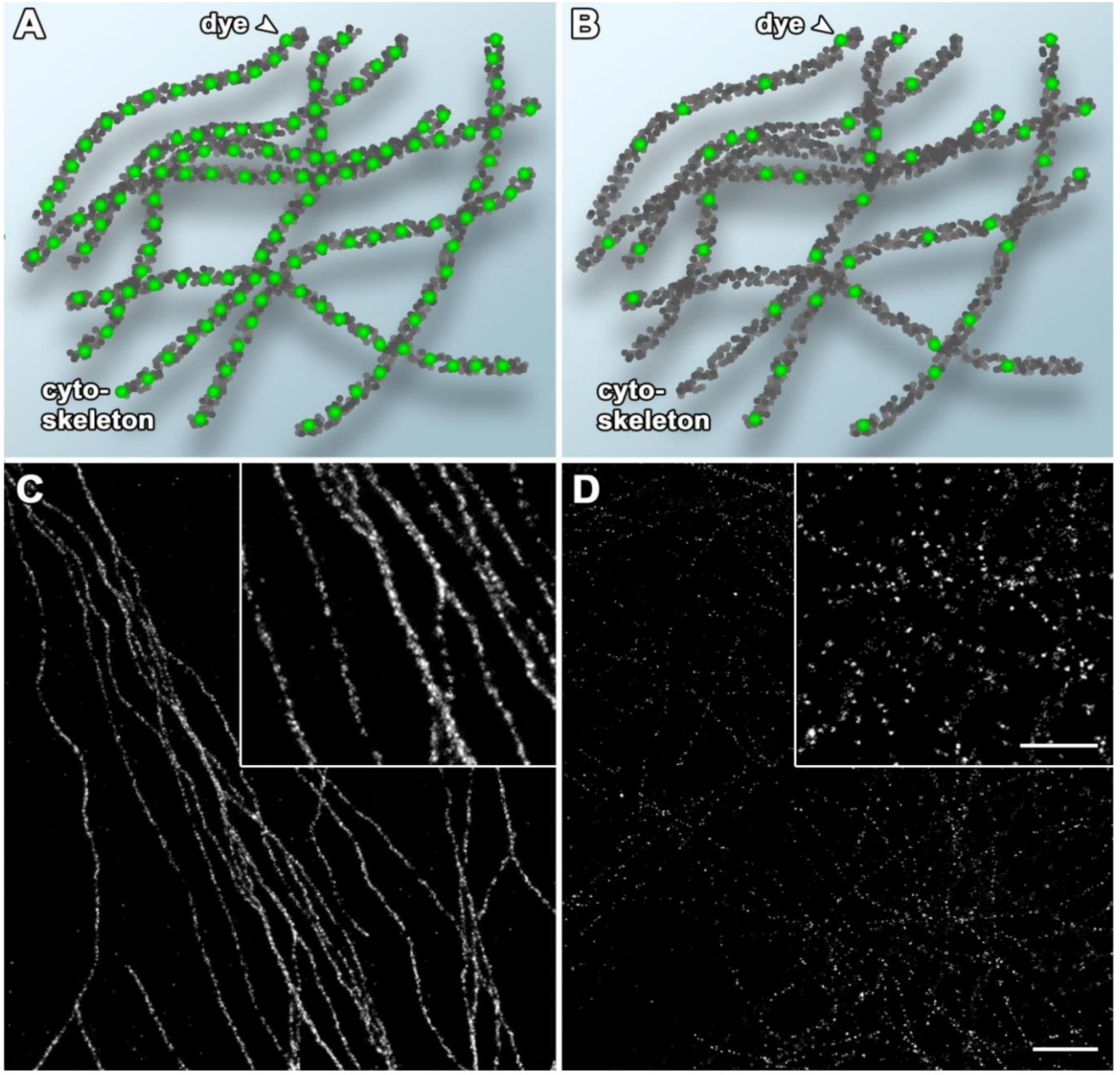
V_HH
15kDa
2nm

IgG complex
>300kDa
20-30nm

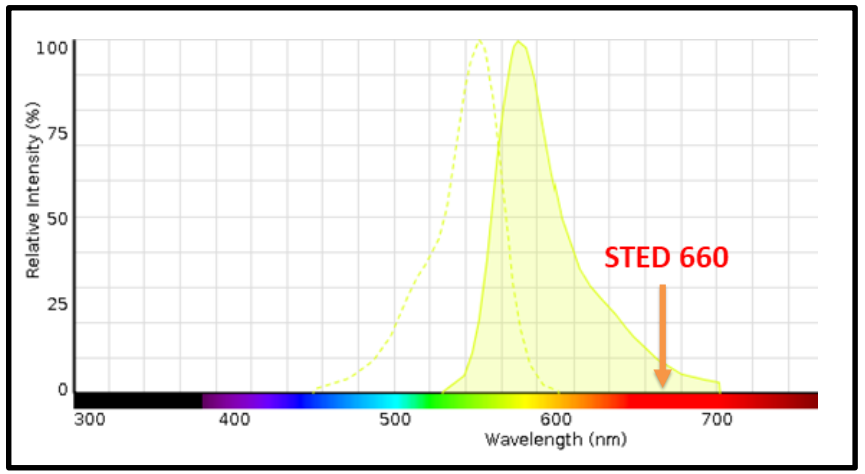
chromotek
new tools for better research

https://www.chromotek.com/technology/discovery-of-nanobodies/

Labeling density in super- resolution



Choice of fluorophores



The ideal fluorophore should have the following characteristics:

1-not be excitable to the wavelength of the STED laser
2-high photostability
3-high probability of de-excitation due to emission stimulated so as to reduce as much as possible the intensity of the laser sted required

	STED 592	STED 660	STED 775
Fixed samples	OG 488	AF 555	STAR635P
	AF 488	ATTO 542	ATTO 647N
Live–cells	Citrine / mVenus	TMR	SiR

Fluorescent label #1			Fluorescent label #2			STED (nm)
Name	Exc. (nm)	Em. (nm)	Name	Exc. (nm)	Em. (nm)	
STAR 440SX	458/470	475 – 510	OG 488	514/520	523 – 580	592
AF 532	532	520 – 565	TMR	580	590 – 650	660
STAR 580	580	600 – 630	STAR 635P	635	655 – 750	775

Tissue clearing strategy for 3D volumetric imaging

The CLARITY techniques removes lipid components, a major light scattering source, form tissues wich are embedded with hydrogel as a structural alternative to lipids.

1.Cut kidney in ~1-2 mm thick chunks and immerse in hydrogel solution

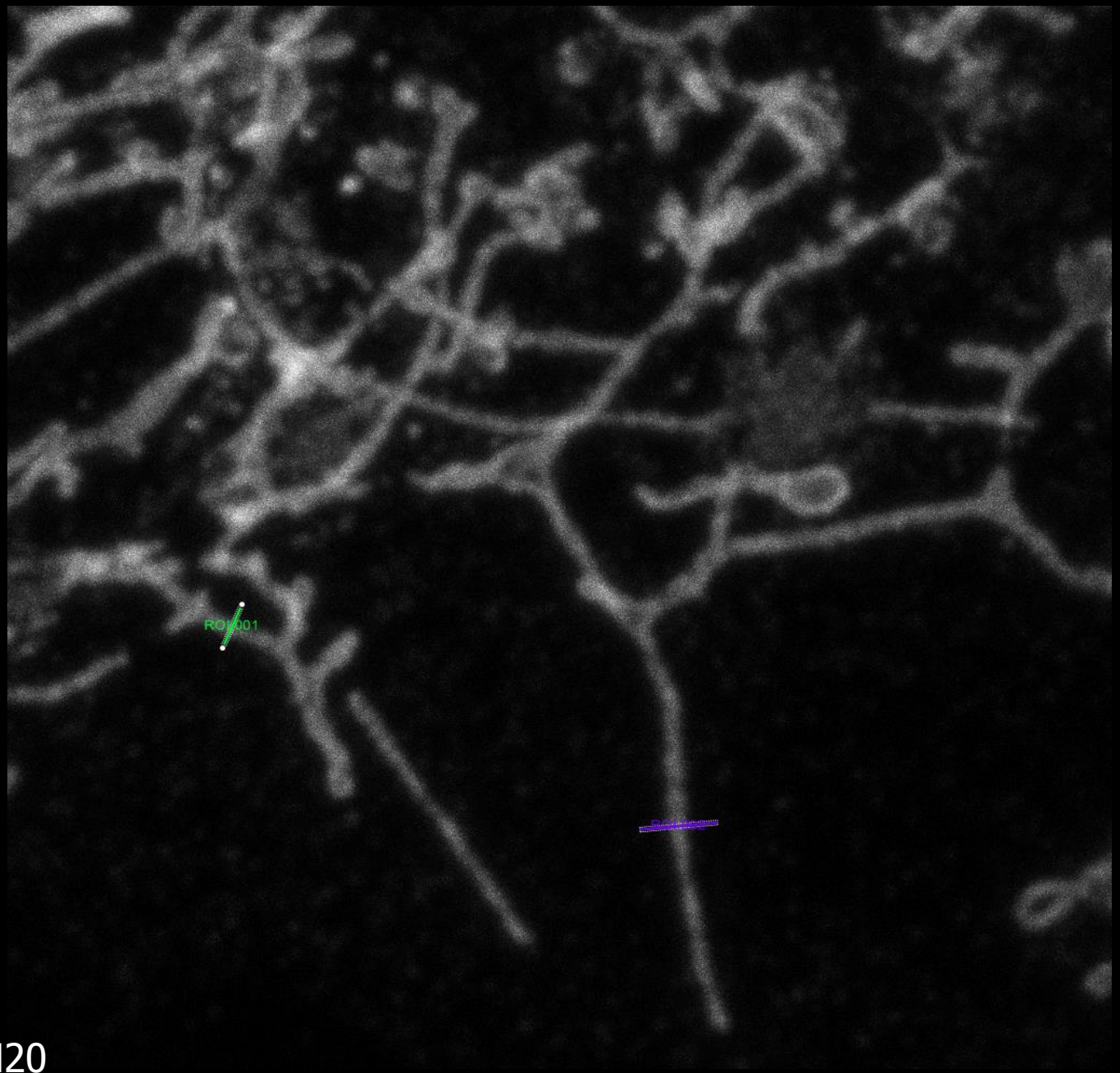
2.Polymerize the gel by incubating at 37° C

3.Embed sample in 3% agarose and cut the sample in 300µm thick slices using a Vibratome

4.Immerse slices in 1-5 mL of clearing solution and incubate at 50° C for 3 days

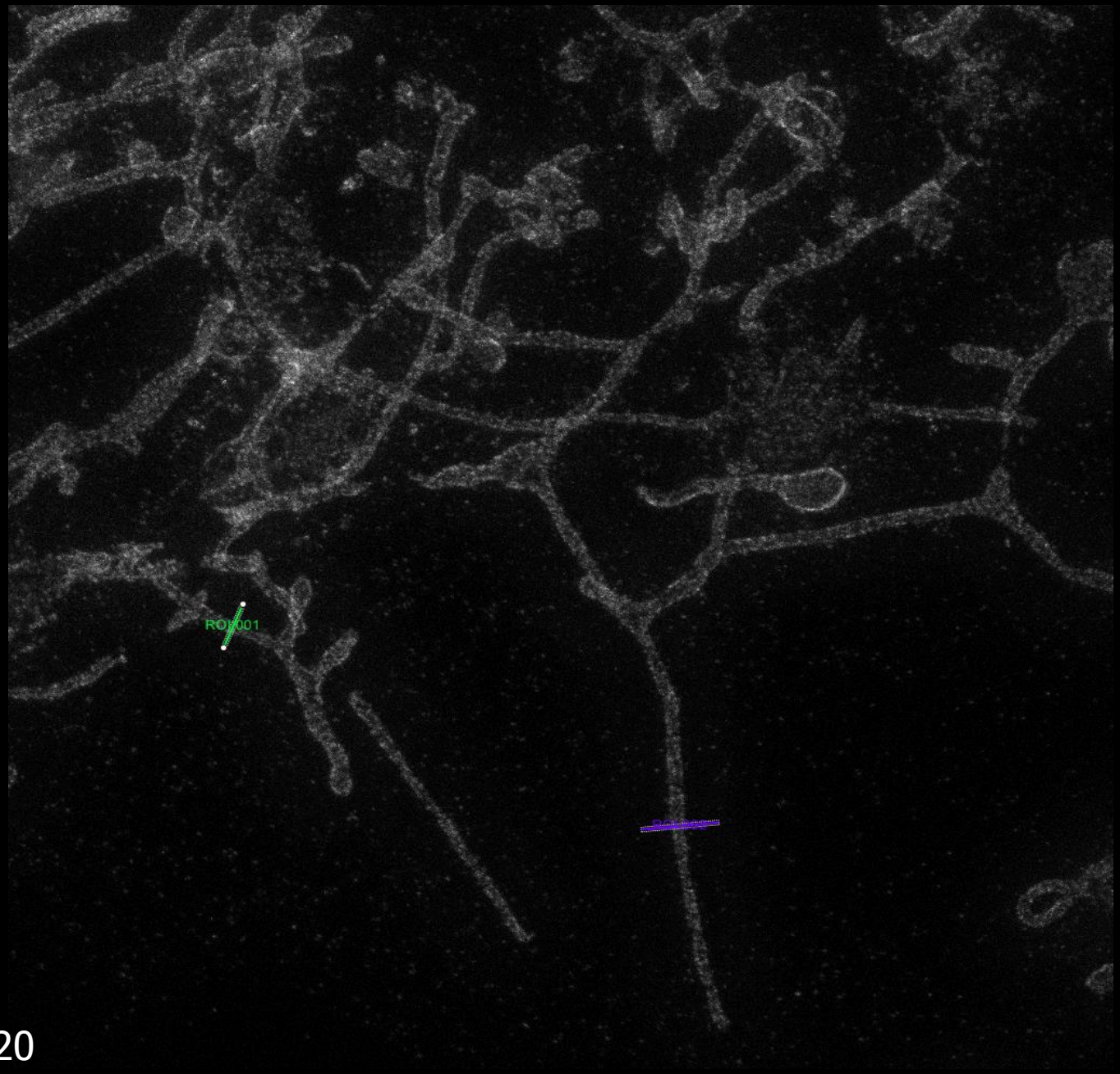
APPLICATIONS…

Studio delle componenti proteiche di membrana e subcellulari



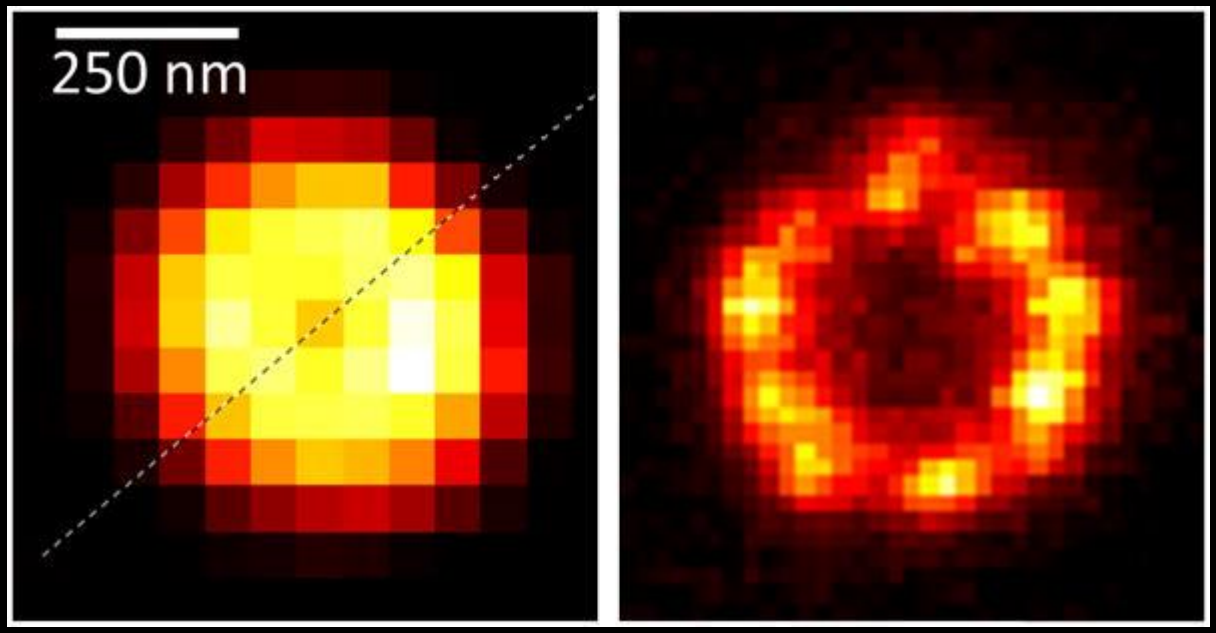
Mitochondria – TOM20

Studio delle componenti proteiche di membrana e subcellulari

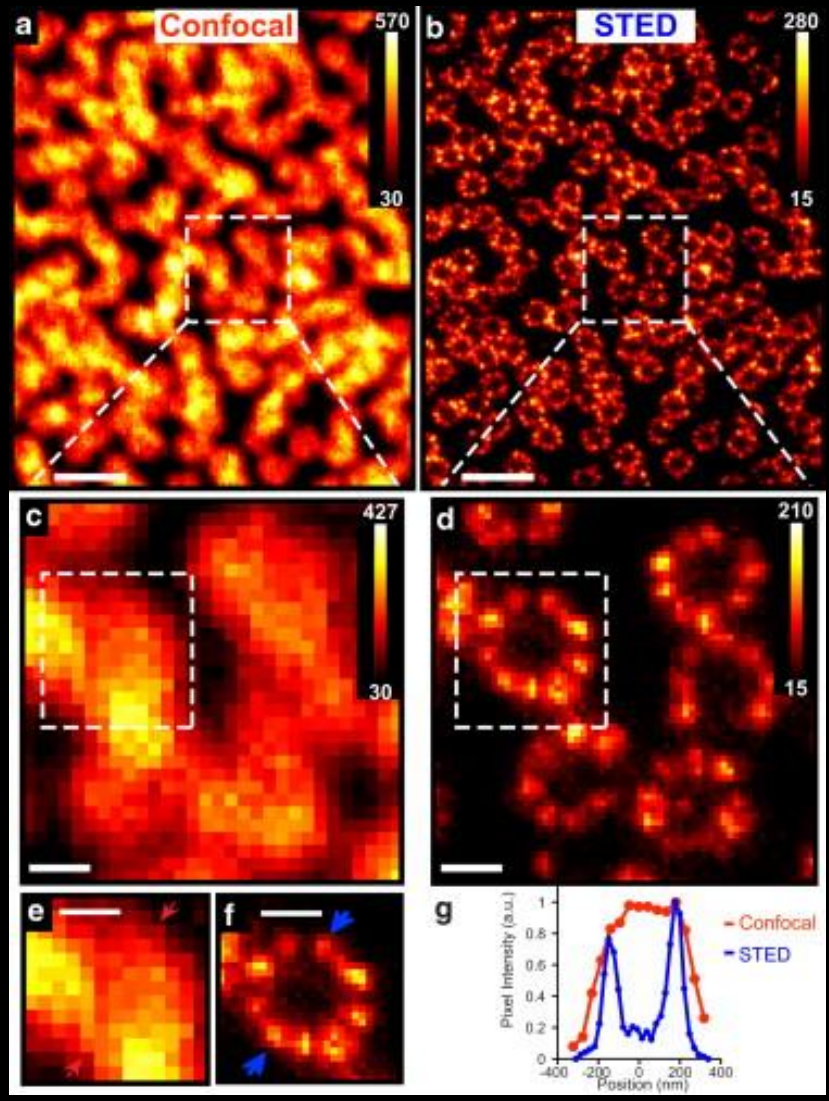


Mitochondria – TOM20

Organizzazione delle subunità dei centrioli

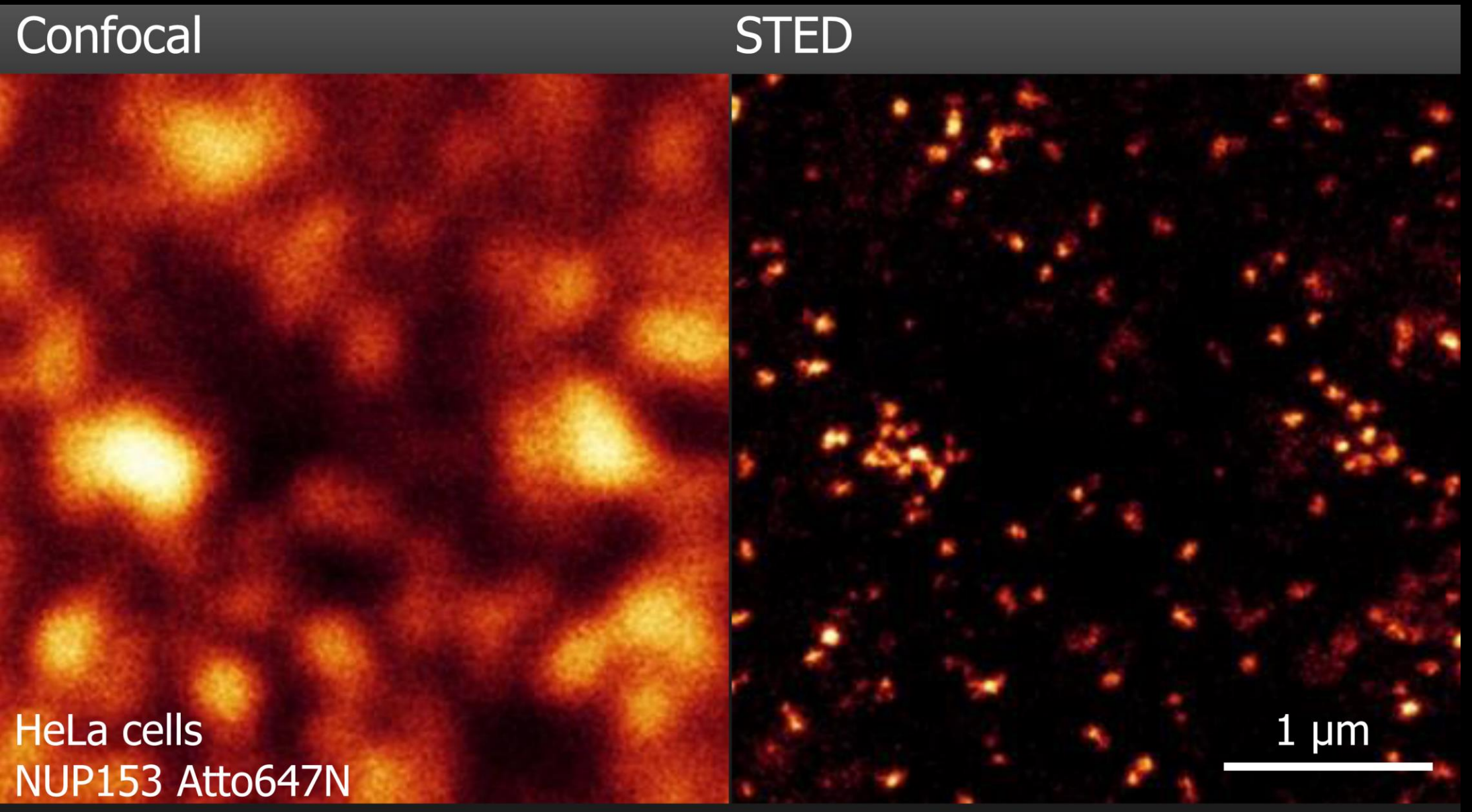


(left) confocal and (right) STED images of Atto647N-IgG immunostained Cep164 in fixed IMCD3 mouse cells. $P_{excitation}$= 10µW, λ_{STED}= 759 nm, P_{STED}= 80mW, 0.5 ms/60 nm pixel (25 nm pixel size for STED).

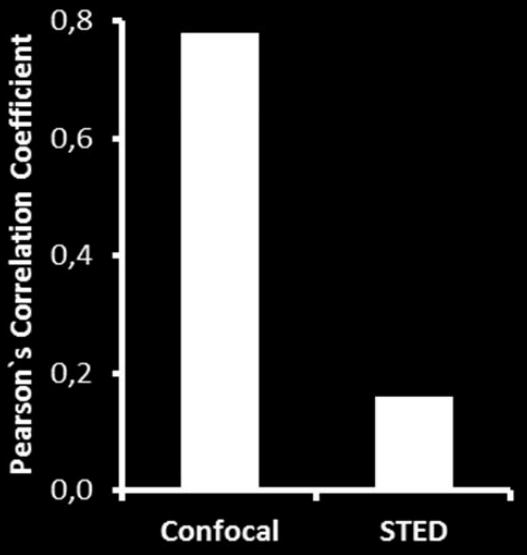


Cep164 is arranged in a nine-fold symmetric pattern around the centriole, consistent with findings suggested by cryoelectron tomography

Organizzazione delle subunità dei nuclear pore complex

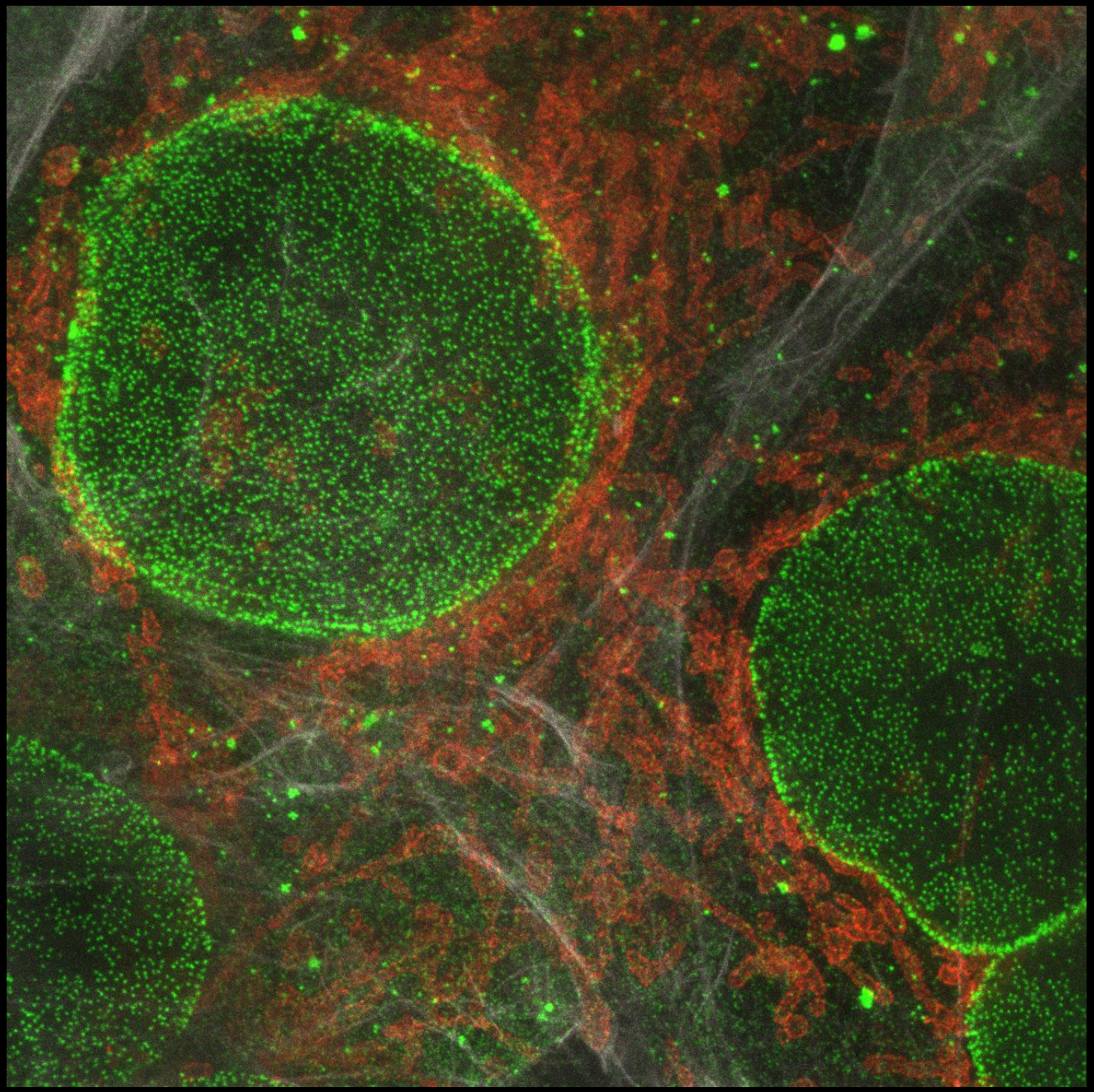


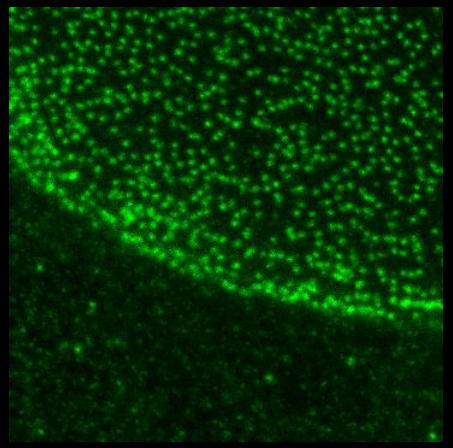
Studi sulla co-localizzazione e clustering delle proteine

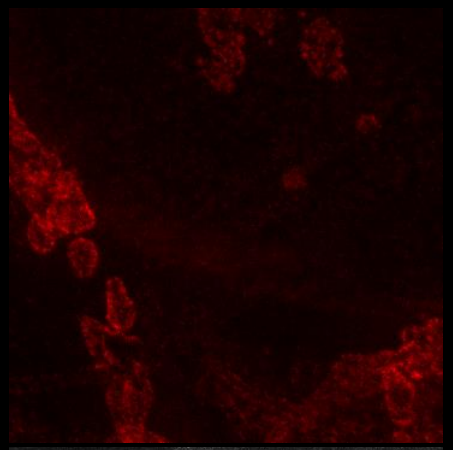


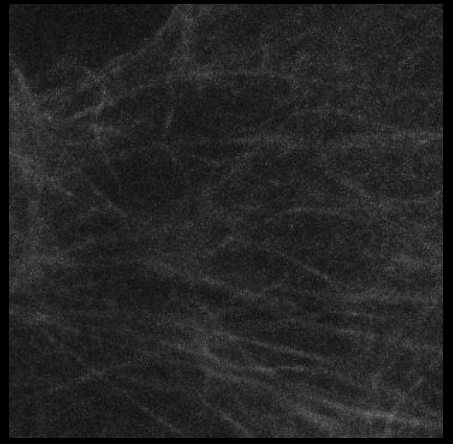
Centrioles in U2OS cells visualized by indirect immunofluorescence. Co-localization of Centrin3 – Alexa Fluor 594 (green) and Cep152 – Alexa Fluor 647 (red) in confocal (left) and deconvolved STED image (right). Sample courtesy of Ella Fung, CRUK/MRC Oxford Institute for Radiation Oncology, UK. The anti-Cep152 antibody was kindly provided by E. Nigg, Biozentrum, University of Basel, Switzerland (Sonne KF et. al. J Cell Science 2013).

Multicolour Imaging: distribuzione delle proteine a livello nanometrico







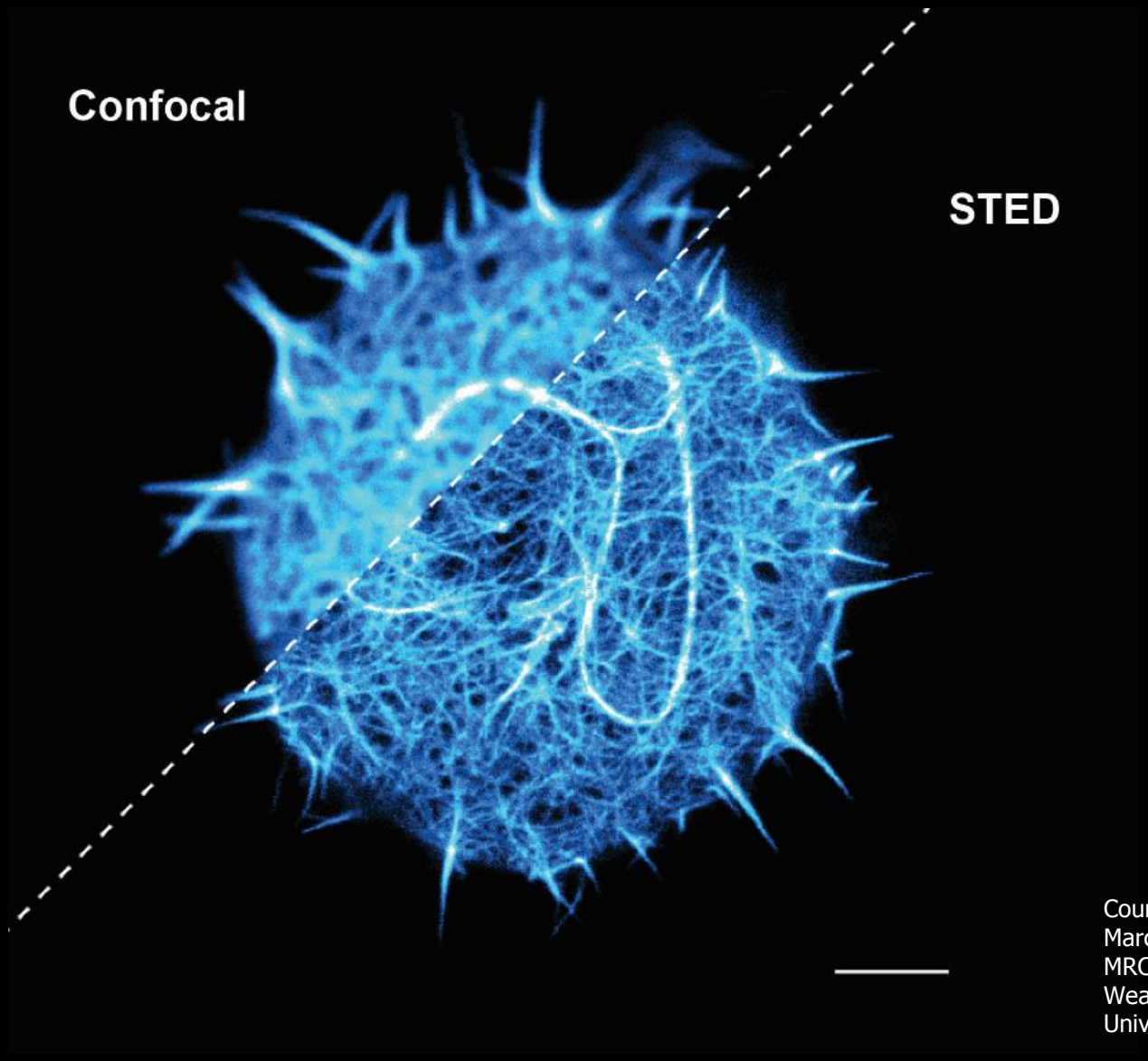


Cos7 cells
Green: AF568 F(ab)' NUP153
Red: Atto594 TOM20
B&W: SiR Actin

Courtesy: Urs Ziegler, Jana Doehner
ZMB, University of Zurich

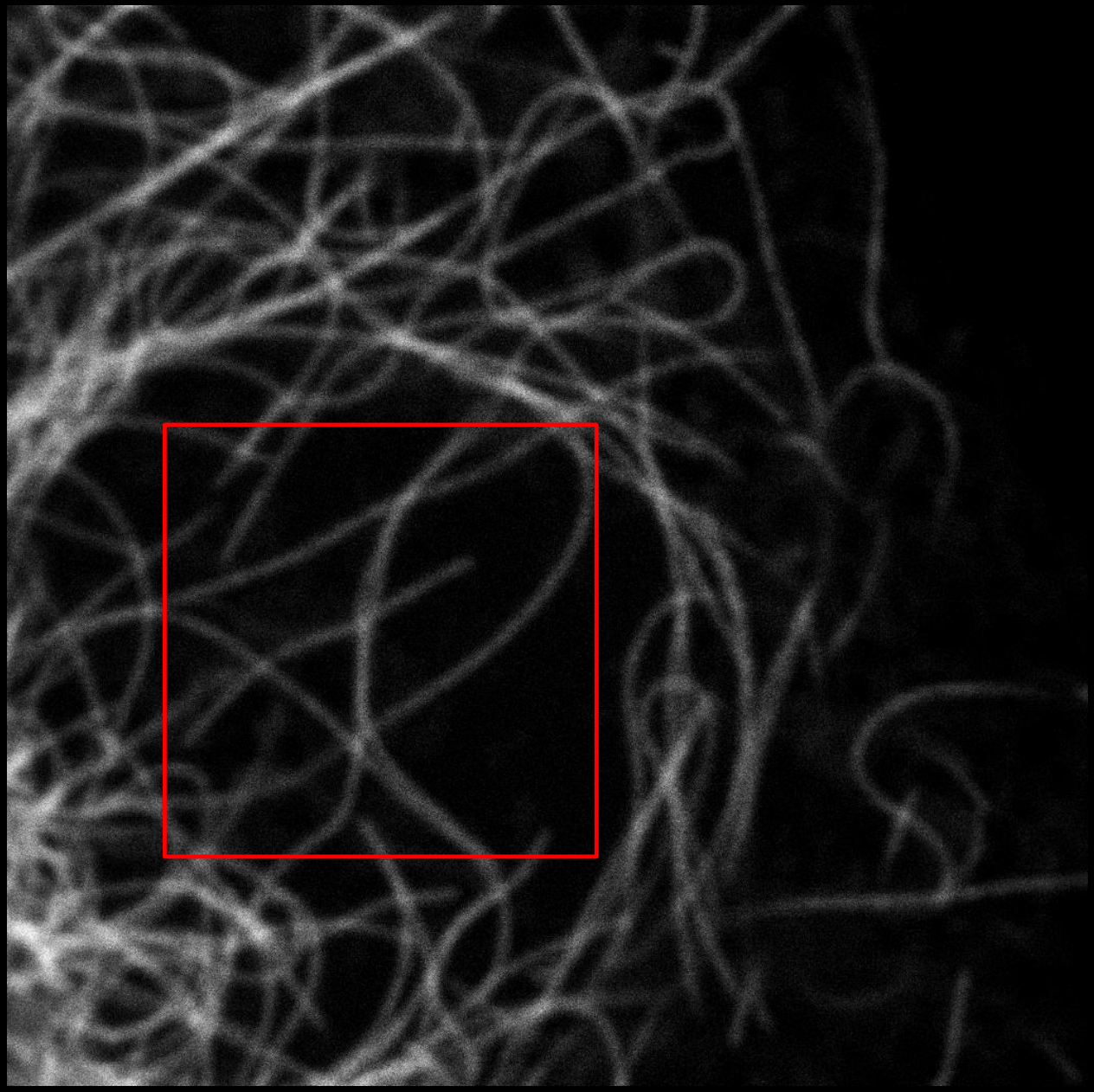
Immunology

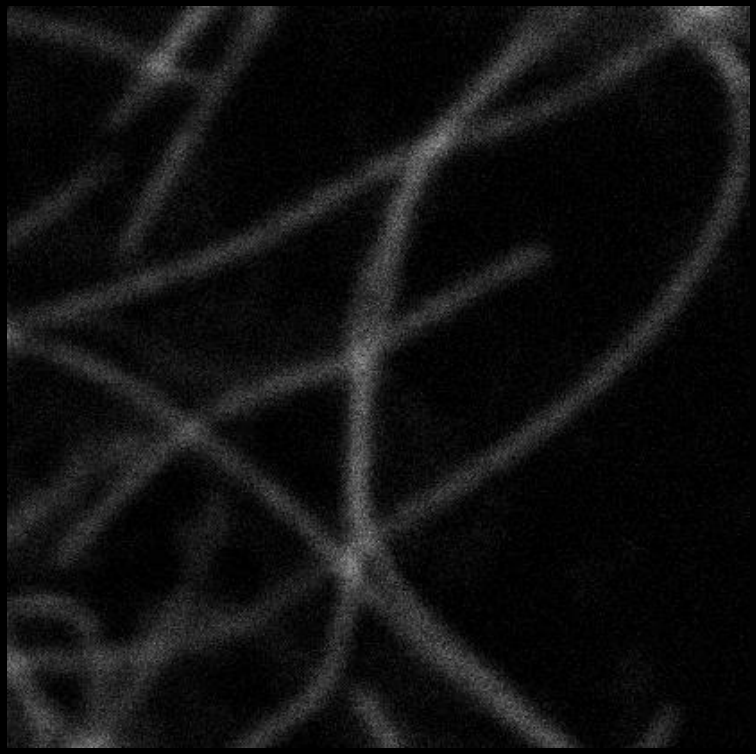
Living T cell in suspension - Actin visualized by Lifeact



Courtesy of
Marco Fritsche, Mathias Clausen and Christian Eggeling
MRC Human Immunology Unit
Weatherall Institute of Molecular Medicine
University of Oxford , UK

Live-Cell Imaging



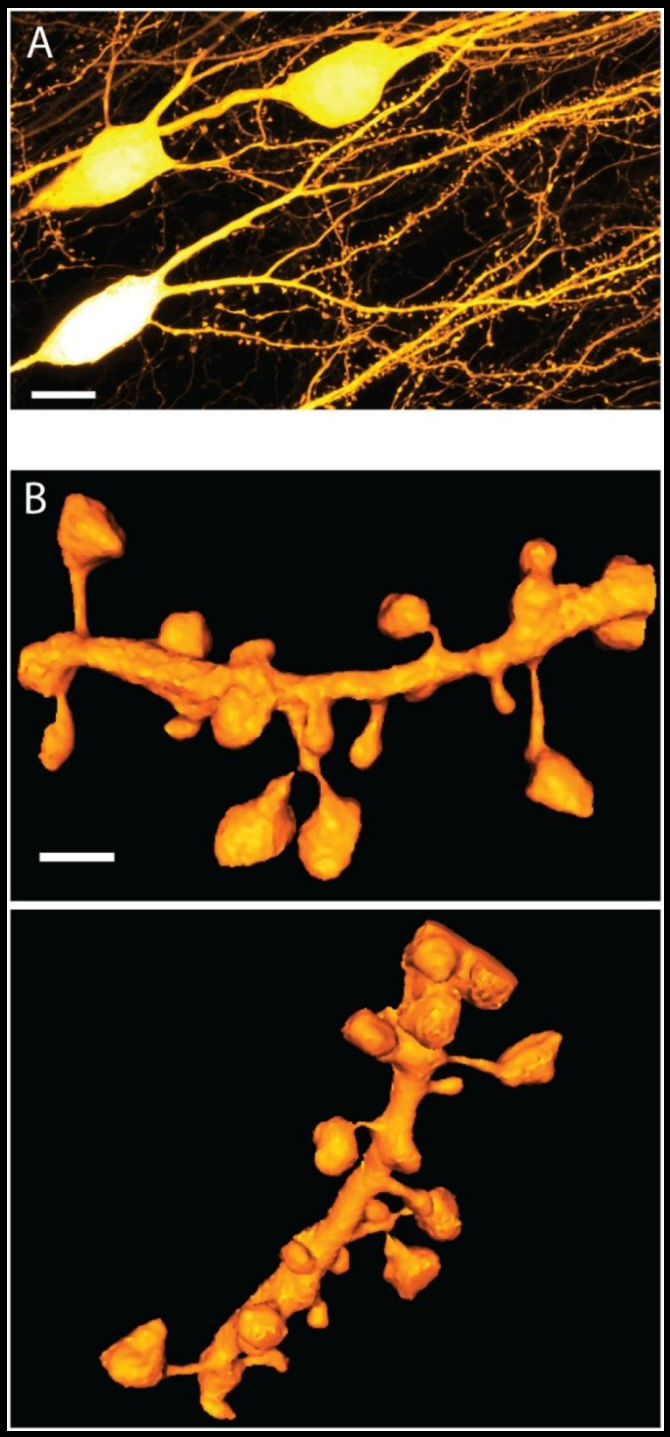


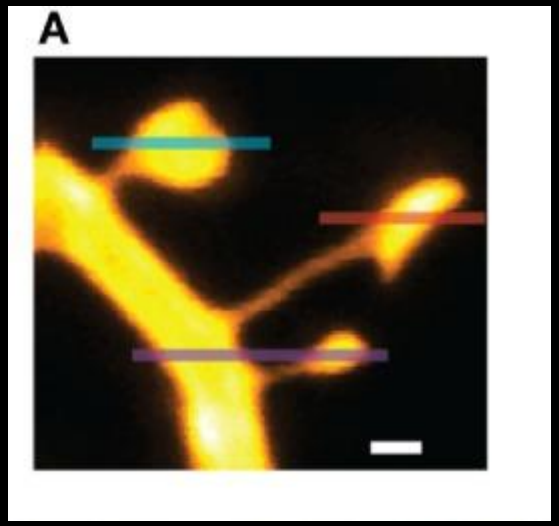
SiR probe courtesy of Kai Johnsson, Grazvydas Lukinavicius
EPFL, Lausanne, Switzerland

Live-Cell Imaging

SiR probe courtesy of Kai Johnsson, Grazvydas Lukinavicius
EPFL, Lausanne, Switzerland

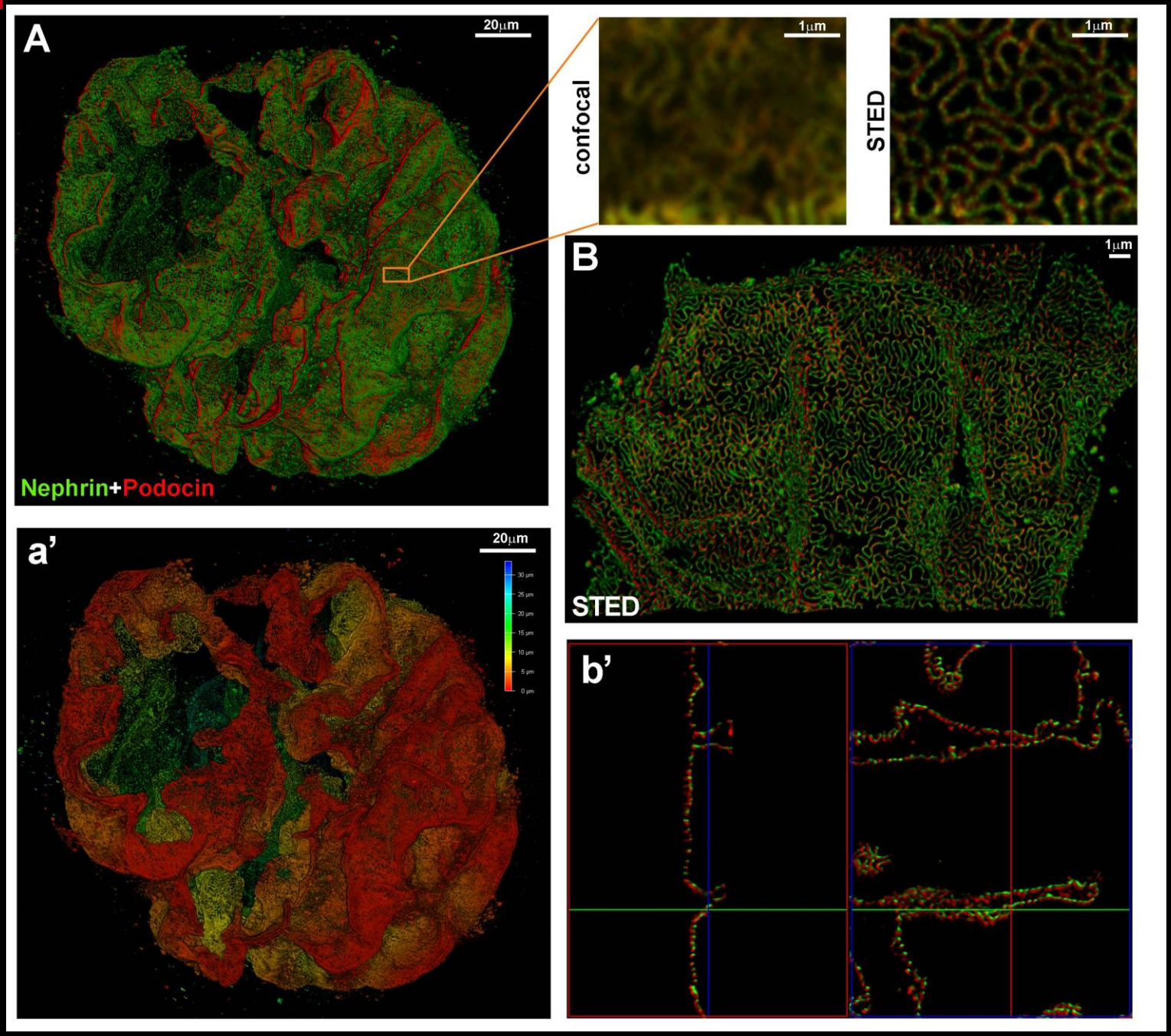
Dendritic Spines

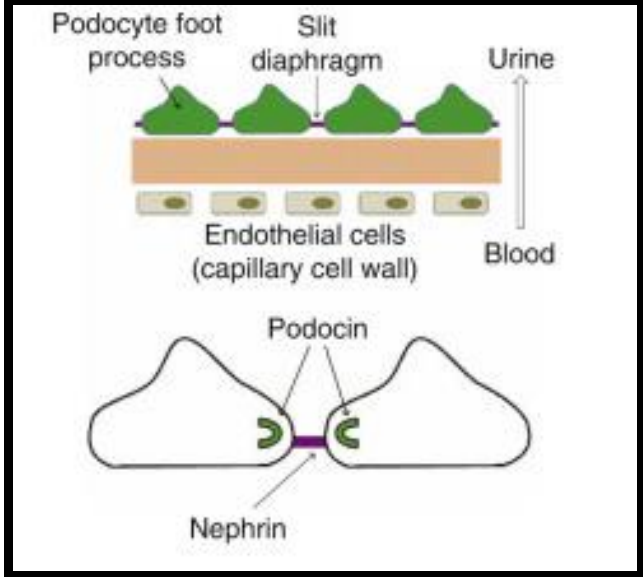




Dendritic spine morphology. **(A)** STED image of basal dendrites on live CA1 pyramidal cells in organotypic hippocampal slice prepared from Thy1-YFP transgenic animals. The image is a maximum intensity projection over 10 µm and is subjected to a 1-pixel median filter. Scale bar is 10 µm.

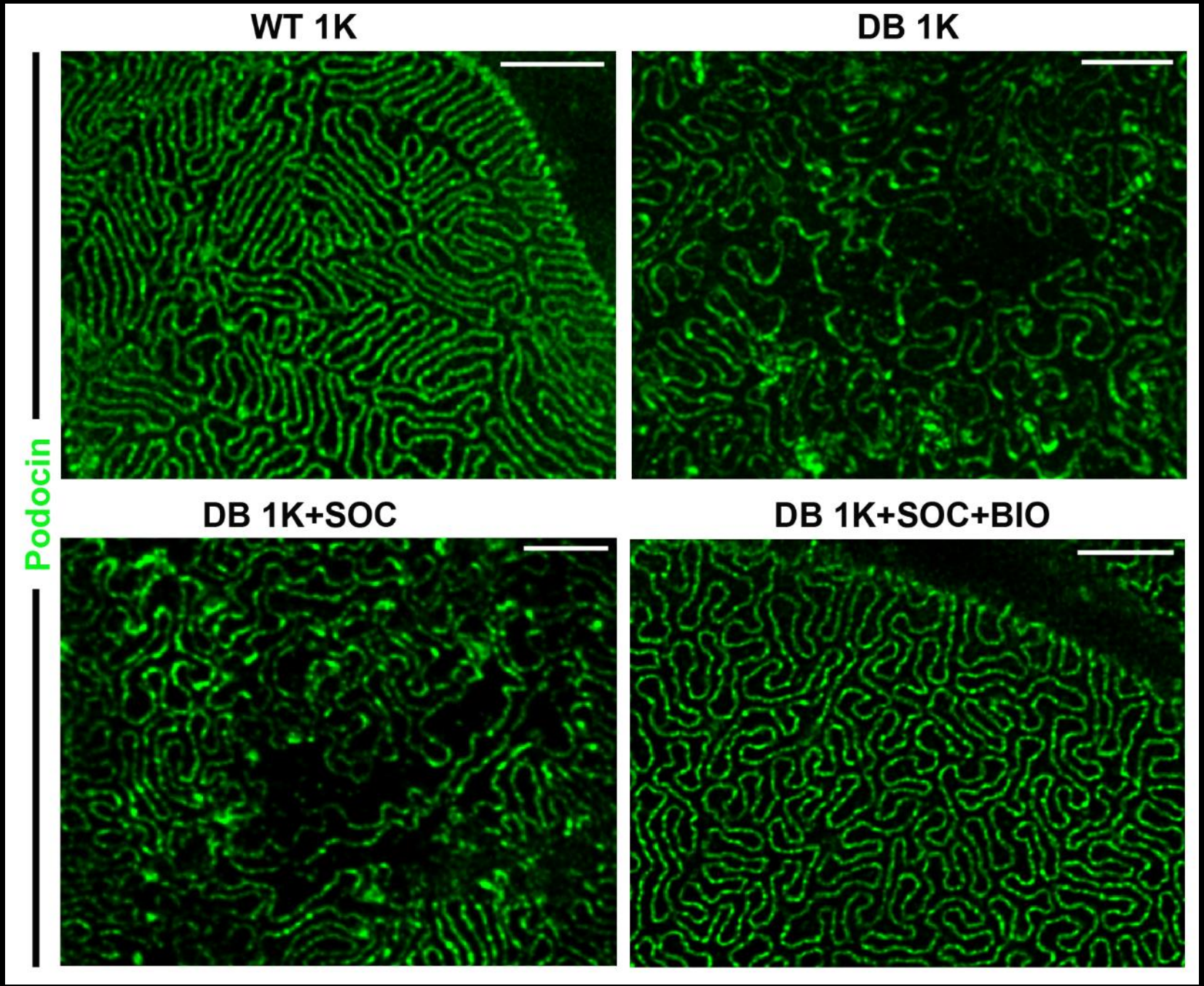
The slit diaphragm in optically cleared kidney tissues



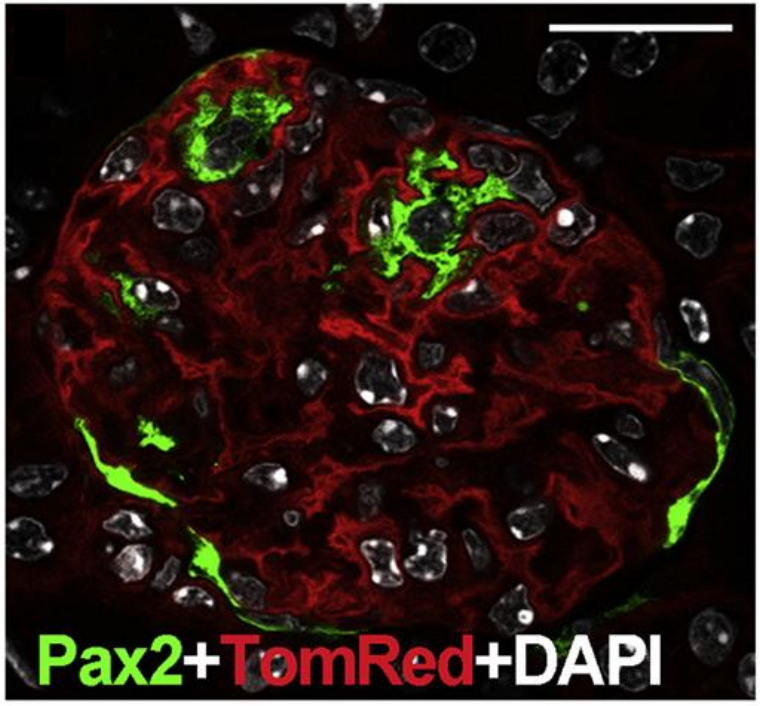


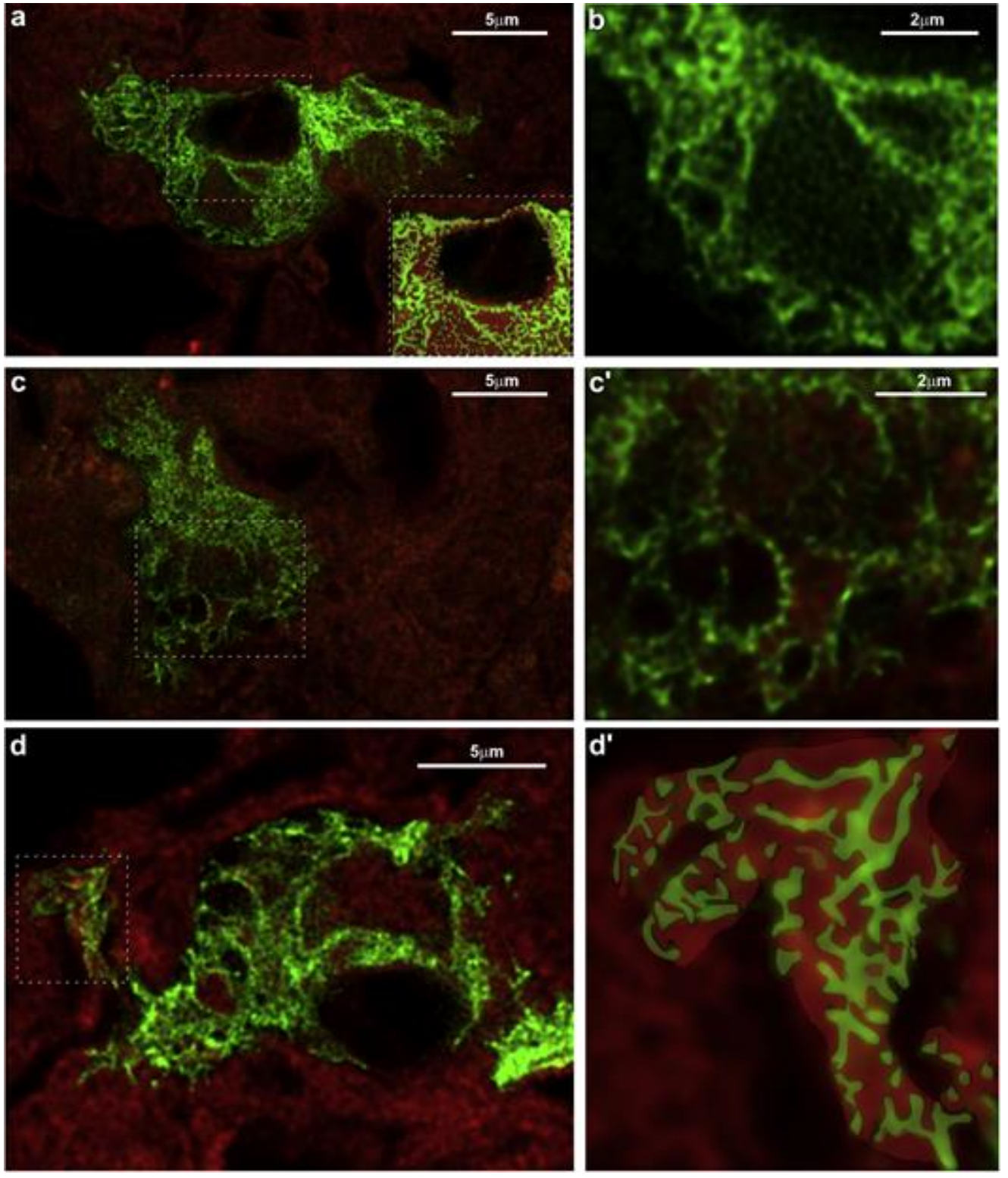
Angelotti ML *et al*. Nephrology Dialysis Transplantation*, 2019*

Study of glomerual filtration barrier in a mouse model of Diabetic Nephropathy



STED super-resolution microscopy reveals new podocytes to fully integrate into the glomerular filtration barrier





Romoli S, Angelotti ML, Antonelli G *et al*, Kidney International,2018

Grazie per l’attenzione!